

PATENT COOPERATION TREATY

by fax and post

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

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PCT/CA 03/00850
VANCOUVER, B.C.NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

Applicant's or agent's file reference
81601-31

IMPORTANT NOTIFICATION

International application No.
PCT/CA 03/00850International filing date (day/month/year)
05.06.2003Priority date (day/month/year)
05.06.2002Applicant
HER MAJESTY IN RIGHT OF CANADA AS REPRESENTED ...

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.
4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 81601-31	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/CA 03/00850	International filing date (day/month/year) 05.06.2003	Priority date (day/month/year) 05.06.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/90		
Applicant HER MAJESTY IN RIGHT OF CANADA AS REPRESENTED ...		



1. This International preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

 These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(II) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 31.12.2003	Date of completion of this report 16.11.04
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Rutz, B Telephone No. +49 89 2399-7828 

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/CA 03/00850

I. Basis of the report

1. With regard to the elements of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-181 as originally filed

Sequence listings part of the description, Pages

1-3 as originally filed

Claims, Numbers

5 (part), 6-23 as originally filed
1-4, 5 (part) filed with telefax on 17.09.2004

Drawings, Sheets

1/15-15/15 as originally filed

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

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5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 7, with regard to IA: 1-4, 18-20 (all partially)

because:

☒ the said international application, or the said claims Nos. with regard to IA: 1-4, 18-20 (all partially) relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 7

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the Standard.

☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-6, 8-23
	No: Claims	-
Inventive step (IS)	Yes: Claims	10
	No: Claims	1-6, 8, 9, 11-23
Industrial applicability (IA)	Yes: Claims	1-4, 18-20 (all partially), 5, 6, 8-17, 21-23
	No: Claims	-

2. Citations and explanations

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see separate sheet

Re Item III

Claims 1-4 and 18-20 inter alia relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT (i.e. method of treatment of the human or animal body). Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

- D1: DATTA H J ET AL: "Intracellular generation of single-stranded DNA for chromosomal triplex formation and induced recombination." NUCLEIC ACIDS RESEARCH. ENGLAND 15 DEC 2001, vol. 29, no. 24, 15 December 2001 (2001-12-15), pages 5140-5147, XP002253387, ISSN: 1362-4962
- D2: J-R MAO ET AL: "Gene regulation by antisense DNA produced in vivo" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 270, no. 34, 25 August 1995 (1995-08-25), pages 19684-19687, XP002132578, ISSN: 0021-9258
- D3: MIROCHNITCHENKO O ET AL: "Production of single-stranded DNA in mammalian cells by means of a bacterial retron" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 269, no. 4, 28 January 1994 (1994-01-28), pages 2380-2383, XP002132577, ISSN: 0021-9258

1. Subject matter

Present application relates to the modification of target nucleic acids in a host genome by homologous recombination with in vivo expressed ssDNA or RNA-DNA hybrids. Said expression is achieved from bacterial retrons which have been transfected into eukaryotic cells (for example yeast). To increase the efficiency of the process a reverse transcriptase was targeted to the nucleus by means of a nuclear localization sequence (NLS).

2. Novelty (Art. 33(2) PCT)

The prior art reports homologous recombination with in vivo expressed ssDNAs (D1). Furthermore, it contains the step of reverse transcription of a gene targeting construct (D1, Fig. 1) and the reverse transcribed sequence is homologous to a target locus and comprises a modification compared to the target nucleic acid. However, the transcript described in D1 is not capable of self-priming reverse transcription, but reverse transcription depends on the presence of tRNAs binding to dedicated primer binding sites (D1, p. 5142, bridging sentence).

Claims 1-6 and 8-23 are considered novel (Art. 33(2) PCT).

3. Inventive step (Art. 33(3) PCT)

Prior art document D1 is considered closest prior art for present application. The difference to present application lies in the use of a different in vivo expression system: reverse transcription from a MoMuLV inverted repeat sequence combined with a restriction enzyme system to release a ssDNA. The technical problem imposed by this difference can be formulated as: provision of a method of in vivo ssDNA expression for homologous recombination which does not depend on tRNA primers for reverse transcription and does not rely on cleavage by a restriction enzyme subsequent to reverse transcription. The solution has been provided in present application with the use of bacterial retrons as expression vectors.

However, this solution cannot be considered inventive because the expression of ssDNAs by retrons in eukaryotic cells to form triple helices has been described in the prior art (D2, p. 19686, last paragraph - p. 19687, first paragraph). Moreover, the person skilled in the art was aware that triple helix forming ssDNA was the gene targeting agent which had been successfully employed in D1 (p. 5144, last paragraph - p. 5145, first paragraph). The combination of D1 and D2 to arrive at the solution of present application was thus obvious for the person skilled in the art.

Claims 1-6, 8, 9 and 11-23 lack inventive step (Art. 33(3) PCT).

The targeting of a reverse transcriptase to the nucleus by means of an NLS was not obvious from the prior art and can thus be considered inventive. An indication relating to the localization of RT is given in D3: "The comparatively low synthesis of msDNA in transfected mammalian cells may be due to highly organized compartmentalization of eukaryotic cells, which may lower the efficiency of RT to form a complex with the primary transcript of the retron." (D3, p. 2382, right column, l. 27-31). However, the person skilled in the art is not provided with a hint how to overcome this problem.

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Claim 10 is considered inventive (Art. 33(3) PCT).

4. Industrial application

For the assessment of the present claims 1-4, 18-20 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

WE CLAIM:

- 1) A method of modifying a target nucleic acid of interest at a target locus within a genome of a host comprising:
- 5 a) introducing into the host a gene targeting construct (GTC) and culturing the host so as to:
- i) express the gene targeting construct encoding a gene targeting RNA, to produce the gene targeting message RNA capable of self-priming reverse transcription by a reverse transcriptase (RT);
- 10 ii) reverse transcribe at least a portion of the gene targeting message RNA to produce an *in vivo* gene targeting substrate (GTS) having a gene targeting nucleotide sequence (GTNS), wherein the GTNS is homologous to the target locus and comprises a sequence modification compared to the target nucleic acid; and,
- 15 b) selecting a host having the sequence modification at the target locus.
- 2) The method of claim 1, wherein the host is capable of expressing the RT prior to transforming the host with the gene targeting construct.
- 20 3) The method of claim 1, wherein the host is modified to be capable of expressing the RT at the same time as, or after, transforming the host with the gene targeting construct.
- 25 4) The method of claim 1, 2 or 3, wherein the GTC is introduced into the host by transformation, by cross breeding or by cell fusion.
- 30 5) A gene targeting construct comprised of recombinant nucleic acid sequences in a host having a host genome, wherein the gene targeting construct encodes and is capable of expressing a gene targeting message RNA, wherein the gene targeting message RNA is capable of self-priming reverse transcription by a reverse transcriptase in the host to produce a gene targeting substrate having a gene targeting nucleotide sequence,